REMARKS

The Office Action

The Combined Declaration and Power of Attorney is objected to. The specification is objected to for containing browser-executable code.

Claims 1, 4, 5, and 12-22 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. All pending claims stand further rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claim 19 stands rejected under 35 U.S.C. § 102(b) as anticipated by Lindvall *et al.* (*Ann. Neurol.* 31: 155-165, 1992).

Combined Declaration and Power of Attorney

A newly executed Combined Declaration and Power of Attorney form is enclosed herewith. This objection can now be withdrawn.

Objection to the Specification

All browser-executable code has been deleted from the specification by amendment. This objection can now be withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner applies a two-part rejection. First, the Examiner asserts that "[t]he specification fails to provide an enabling disclosure for the genetic modification of human ES cells" because success in mouse ES cells is not predictive of success in human ES cells. Second, the Examiner asserts that the production of replacement neurons of the desired phenotype at critical locations is not enabled. Each of these bases of rejection is addressed separately.

Genetic Modification of Human ES Cells

The Examiner asserts that the specification is not enabling because successful genetic modification of mouse ES cells is not predictive of success in human ES cells. To support this rejection, the Examiner points out that Zwaka *et al.* (*Nature Biotechnol.* Advance Online Publication, February 10, 2003; "Zwaka") recently demonstrated significant differences in transfection efficiencies between mouse and human ES cells. The Examiner also notes a recent study by Eiges *et al.* (*Curr. Biol.* 11: 514-518, 2001; "Eiges") which compares the efficiency of different transfection protocols for human ES cells. The Examiner argues that, together, these studies prove that methods for successfully transfecting human ES cells were not available at the time of application filing. Applicants respectfully disagree.

Applicants respectfully submit that the Examiner's reading of Zwaka is unduly narrow and does not demonstrate that the present specification is non-enabling. The Examiner characterizes Zwaka as teaching that "[h]igh, stable transfection efficiencies in human ES cells have been difficult to achieve, and, in particular, electroporation protocols established for mouse ES cells work poorly in human ES cells." *Office Action* mailed February 26, 2003, page 4, fifth paragraph; emphasis added.

Applicants first point out that mere inefficiency is not an appropriate basis for a lack of enablement rejection. In the statement relied upon by the Examiner, Zwaka refers to transfecting human ES cells using protocols that had been established and optimized for mouse ES cells. Zwaka discloses the mouse ES cell protocols yielded a stable transfection rate of about 10^{-7} in human ES cells. *Zwaka*, page 1, right column, first paragraph. Although Zwaka suggests that murine-optimized electroporation protocols work poorly in human ES cells and that higher transfection rates are desirable, nowhere does Zwaka suggest that electroporation of human ES cells is entirely unsuccessful. Thus, for this reason alone, Zwaka proves that the instant specification enables the genetic modification of human ES cells.

Zwaka also demonstrates that, by performing nothing more than routine experimentation, the murine electroporation protocols may be optimized for human ES cells and higher yields obtained. Specifically, Zwaka states that

> [a]s human ES cells are significantly larger than mouse ES cells (~14 μm versus ~8 μm), we tried electroporation parameters described for larger cells. Additionally, we electroporated the cells in an isotonic, protein-rich solution (standard cell culture medium), instead of PBS, at room temperature. Using this modified protocol, we were able to obtain stable ... transfection rates that were 100-fold (or more) higher than those attained with standard mouse ES cell electroporation procedures. Zwaka at page 1, right column, first paragraph.

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> [e]lectroporation of human ES cells with a DNA construct containing a *neo* cassette under the control of the *tk* promoter yielded a stable transfection rate of 5.6×10^{-5}, giving an estimated 26:1 ratio of stable transfected clones to homologous recombination events for the first POU5F1 construct. Similarly, for transfection of the HPRT1 vector, the ratio of G418-resistant clones to $HPRT1^-$ clones was 50:1. Zwaka at page 2, first paragraph (citation omitted) (emphasis added).

Zwaka, using standard techniques known in the art at the time of application filing, easily modified the murine-optimized electroporation protocols to increase transfection efficiency in human ES cells.

Zwaka further provides the artisan with an expectation of success by teaching that the recombination event is likely equivalent in mouse and human ES cells. Zwaka notes that "although successful transfection strategies differ between human and mouse ES cells, the frequency of homologous recombination itself may be similar." Zwaka at page 2, first paragraph; emphasis added.

In sum, Zwaka demonstrates that the results of murine-optimized electroporation protocols are successful, albeit inefficient, when applied to human ES cells. Zwaka further demonstrates that the protocols are easily improved by routine experimentation using techniques known in the art for transfection of larger ES cells (i.e., human). And finally, Zwaka confirms that the process of homologous recombination is similarly

efficient in human and murine ES cells (not that efficiency is relevant to enablement under § 112). Accordingly, when read in its entirety, Zwaka alone demonstrates the viability of creating genetically modified human ES cells using the electroporation technique and proves that Applicants' specification enabled genetic modification of human ES cells at the time of filing.

Turning next to Eiges *et al.*, the Examiner points out that the transfection efficiency of several protocols is compared. Specifically, Eiges compares three chemical transfection reagents (Lipofectamine, FuGENE, and Exgen 500 (polyethylenimine)) and electroporation. Eiges, like Zwaka, demonstrate that electroporation of human ES cells is successful (Eiges, Figure 1). Eiges also demonstrates that transfection of human ES cells using FuGENE (Boehringer Mannheim) and ExGen 500 (Fermentas, Inc.) is successful. Although it is of course true, as the Examiner states, that Eiges had not been published at the time of application filing, this is irrelevant because the relevant methods used by Eiges, transfections using FuGENE and ExGen 500 (polyethylenimine) were routine in the art at the time the application was filed. See, for example, Remy *et al. Adv. Drug Deliv. Rev.* 30: 85-95, 1998; Uyttersprot *et al. Mol. Cell. Endocrinol.* 142: 35-39, 1998; Wiesenhofer *et al. J. Neurosci. Meth.* 92: 145-152, 1999; copies enclosed.

In sum, the utility of the electroporation technique on human ES cells is demonstrated by Zwaka and Eiges demonstrates that human ES cells may be successfully transfected using well known chemical reagents and techniques (i.e., FuGENE, ExGen 500, and electroporation). The fact that these references are post-filing art does not negate their use for proving that the present specification enables the claimed invention. Accordingly, the art cited by the Examiner proves that the specification, combined with techniques and reagents available at the time of filing, enables the genetic modification of human ES cells.

Embryonic Stem Cell Transplantation

The Examiner asserts that the specification does not enable the treatment of Parkinson's Disease (PD) because "the transplanted cells must produce replacement neurons at critical locations" and that "these replacement neurons must have the appropriate phenotype necessary to correct the deficiency." *Office Action*, paragraph bridging pages 4 and 5. In applying this rejection, the Examiner finds unpersuasive the Declaration by Dr. Isacson (mailed December 2, 2002; "Isacson Declaration") because the disclosed results use transplanted murine ES cells in a mouse model of PD, whereas "the claimed invention is directed to treatment of Parkinson's disease, a disease of humans." *Office Action*, page 5, first paragraph. The Examiner concludes therefore that "results obtained using mouse ES cells are not considered predictive of results that can be achieved in humans, using the same protocols." *Office Action*, page 5, first paragraph. Applicants respectfully traverse this ground of rejection.

Transplanted Cells Produce Replacement Neurons

The Examiner first argues that "the state of the art for *in vivo* differentiation of ES cells is undeveloped" and that "little is known about the behavior of these cells *in vivo* or how they will interact with the local environment when implanted into adult tissues." *Office Action*, page 5, paragraph bridging pages 4 and 5. Applicants respectfully disagree.

At the time of application filing it was known that ES cells, when transplanted into the brain, spontaneously adopt a neuronal phenotype. Deacon *et al.*, Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp. Neurol.* 149: 28-41, 1998; art of record. Further, when transplanted into a striatum having a 6-hydroxydopamine (6-OH-DA) lesion, the ES cells differentiated into dopaminergic and serotonergic neurons and axonal outgrowth from the ES cell graft into the surrounding striatum was observed. See, Deacon *et al.*, Figures 6 and 7. Thus, at the time of application filing, the artisan had an expectation that centrally transplanted ES

cells would differentiate into neuronal phenotypes and innervate the surrounding nervous tissue.

Fetal neurons isolated from the ventral mesencephalon (VM cells) contain, *inter alia*, dopaminergic neurons and are substantially more differentiated than ES cells. Nonetheless, at the time of application filing, it was recognized that VM cell grafts implanted into the human putamen[1] alleviated the clinical symptoms caused by PD and, like transplanted ES cells, innervate the surrounding brain tissue. Lindvall *et al. Ann. Neurol.* 31:155-165, 1992 (art of record); Linvall, *Mov. Disord.* 13(Suppl. 1): 83-87, 1998 (copy enclosed).

When read together, Deacon *et al.*, Lindvall *et al.*, and Lindvall demonstrate that ES cells and fetal VM cells (including fetal dopaminergic neurons) adopt neuronal phenotypes and innervate the surrounding nervous tissue when transplanted into the damaged striatum (caudate, in humans). Further and as noted below, the evidence from human PD patients and non-human animal PD models (including the 6-OH-DA model) prove the effectiveness of dopaminergic cell replacement therapies for the treatment of PD.

The present invention provides a method for treating PD by transplanting genetically modified ES cells that have been lineage-restricted to become dopaminergic neurons. Thus, the cells used in the present invention are not totipotent, as are the ES cells of Deacon, nor are they fully differentiated into dopaminergic neurons. However, when the prior art is viewed in its entirety, a skilled artisan at the time of application filing would, based on a reading of the present specification, expect the modified ES cells to integrate into the nervous tissue at the site of implantation and to innervate the surrounding tissue. And, as discussed further below, Kim *et al.* (*Nature* advance online publication, 20 June 2002) prove that ES cells transfected with Nurr-1 form dopaminergic neurons when transplanted into striatum of a 6-OH-DA-lesioned animal. In sum, the pre- and post-filing evidence demonstrates that the genetically modified ES cells described in

1 The caudate and putamen of primates (i.e., humans) is equivalent to the corpus striatum of rodents.

the instant specification produce replacement neurons at critical locations (i.e., the site of transplantation).

The Specification Enables the Treatment of Parkinson's Disease

Applicants respectfully submit that the Examiner inappropriately dismisses the Declaration of Ole Isacson Under 37 C.F.R. § 1.132 (mailed December 2, 2002; "Isacson Declaration") as unpersuasive and that the Examiner provides no credible evidence that ES cells transplanted in a murine model of PD is not predictive of treatment of the disease in humans.

Applicants note that human testing is not required, for enablement purposes, to support claims of an *in vivo* utility of a biomedical invention. The Federal Circuit has repeatedly stated (*Scott v. Finney*, 34 F.3d 1058,1063 (Fed. Cir. 1994), affirming *In re Watson*, 517 F.2d 465, 476 (C.C.P.A. 1975) and *In re Sichert*, 566 F.2d 1154, 1160 (C.C.P.A. 1977)):

> Title 35 does not demand that such human testing occur within the confines of Patent and Trademark Office (PTO) proceedings ... Congress has given the responsibility to the FDA, not to the [PTO], to determine ... whether drugs are sufficiently safe.

The Federal Circuit, in reversing a Board of Patent Appeals and Interferences decision that *in vitro* data did not support *in vivo* biomedical applications, stated (*In re Brana*, 51 F.3d 1560, 1568 (Fed. Cir. 1995)):

> The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans.

Accordingly, evidence from sources other than human efficacy trails is acceptable when patenting methods and compositions useful for treating human disease.

The Isacson Declaration, in addition to characterizing murine ES cells genetically modified using the methods described in the specification, points to a recent publication

which demonstrates that murine ES cells transfected with Nurr-1 reverses behavioral defects in a murine model of PD (Kim *et al. Nature* advance online publication, 20 June 2002; Exhibit D of Isacson Declaration). Specifically, and as noted in the Isacson Declaration, Kim *et al.* transplanted Nurr-1-expressing ES cells into the striatum of mice unilaterally lesioned using 6-hydroxydopamine (6-OH-DA). The transplanted ES cells adopted a dopaminergic phenotype as measured by electrophysiology and immunohistochemistry. Additionally, the behavioral symptoms of the 6-OH-DA lesion, a surrogate for the symptoms of PD in humans, were reversed. Thus, the study of Kim *et al.*, using lineage restricted, Nurr-1-expressing ES cells equivalent to the genetically modified ES cells described in the present specification, provides an expectation that the claimed method will be successful and demonstrates that the specification fully enables the claimed invention.

To dismiss the findings of Kim *et al.* as non-predictive of PD in human patients is to disregard over thirty years of characterization and correlation of the 6-OH-DA model with the human condition. See, for example, Cashin *et al.* The effect of anti-Parkinson drugs on catalepsy induced by met-p-tyrosine in rats pretreated with intraventricular 6-hydroxydopamine. *Br. J. Pharmacol.* 47: 658P-659P, 1973; Ungerstedt *et al.* Behavioral, physiological, and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. *Adv. Neurol.* 5: 421-426, 1974 (copies enclosed). The 6-OH-DA model has been used extensively in neurobiology and drug screening studies and has been validated in species ranging from rodents (i.e., rats and mice) to non-human primates. See, for example, Gerlach *et al.* Animal models of Parkinson's disease: an empirical comparison of the phenomenology of the disease in man. *J. Neural Transm.* 103: 987-1041, 1996 (copy enclosed). The Examiner has provided no evidence that the 6-OH-DA model is unreliable or inappropriate for testing potential PD therapies.

In sum, Applicants respectfully submit that, at the time of application filing, a skilled artisan, practicing no more than routine experimentation, could have created the

genetically-modified ES cells used in the present invention. The genetically-modified ES cells, when transplanted into the brain of a PD patient, form replacement neurons in critical locations and adopt the appropriate phenotype necessary to correct the deficiency, as disclosed in the specification. For the foregoing reasons, Applicants submit that the specification fully enables the practice of the claimed invention and this rejection should be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

All pending claims stand further rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. The Examiner asserts four distinct rejections and each is addressed separately.

First, the Examiner asserts that the claims are indefinite because the preamble recites "a method of treating a human patient," but no particular treatment effect is achieved. Applicants respectfully traverse this rejection.

There is nothing indefinite about "treating a human patient." "Treatment," when used in a medical context, means "medical ... management of a patient" including, for example, active treatment (directed specifically toward a cure), causal treatment (directed toward removal of the cause of the disease), and palliative treatment (designed for relief of symptoms rather than a cure). *Taber's Cyclopedic Medical Dictionary*, F.A. Davis Co., Philadelphia, PA, 2001, page 2232; copy enclosed. A person of ordinary skill immediately recognizes that a medical treatment may be administered to effect a variety of outcomes (i.e., cure or palliation), each of them medically useful. Thus, "treating a human patient" has a definite and the art-recognized meaning.

Second, the Examiner asserts that the term "cells which are lineage-restricted to dopaminergic neurons" is indefinite because it is unclear whether the term encompasses dopaminergic neurons or only precursor cells that have not yet differentiated into dopaminergic neurons. Although Applicants do not agree that the identified terms are indefinite, in order to expedite prosecution Applicants have amended claims 1 and 14 to

remove the terms. Claim 19 has been amended to recite "recombinant progenitor cells which are lineage-restricted to dopaminergic neurons." Support for this amendment is found in the Specification at page 3, lines 2-4.

Third, the Examiner rejects all pending claims because the term "said lineage-restricted cells of step (c)" lacks antecedent basis. This rejection has been overcome by the present claim amendments.

Fourth, the Examiner rejects all pending claims because, as the Examiner correctly points out, one would not transfect a cell with a protein. This rejection has been overcome by the present claim amendments.

Applicants respectfully submit that all rejections under 35 U.S.C. § 112, second paragraph should be withdrawn.

Rejections Under 35 U.S.C. § 102

Claim 19 stands rejected under 35 U.S.C. § 102(b) as anticipated by Lindvall *et al.* (*Ann. Neurol.* 31: 155-165, 1992). Applicants respectfully point out that claim 19 has been amended to require that the cells are recombinant cells. Lindvall, by contrast, transplanted isolated fetal ventral mesencephalic (VM) cells into PD patients. The VM cells of Lindvall were not genetically modified. Therefore, Lindvall does not anticipate claim 19 as presently amended. Accordingly, this rejection should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is requested. Enclosed is a petition to extend the period for replying for three months, to and including August 26, 2003. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: Aug. 26, 2003

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